Studies on Pathogenic Porcine Enteroviruses

II. Isolation of Virus in Tissue Culture from Brain and Feces of Clinical Cases

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Preliminary studies on a disease of suckling pigs seen in Ontario has suggested it to be in the nature of a virus infection (1, 2). In Britain, Harding *et al* (3) have reported the occurrence of a virus in pigs suffering from an encephalitis identified there as Talfan disease. Serological studies in tissue culture indicated Talfan disease to be related to Teschen disease (4).

Betts (5), also in Britain, successfully isolated enteroviruses from the feces and tonsils of apparently healthy swine. These viruses proved to be pathogenic for colostrum-deprived, pathogen-free piglets, and were serologically distinct from either Talfan or Teschen disease viruses (6).

In this laboratory, previous studies have shown that available strains of European Teschen disease virus grow readily in monolayer cultures of pig kidney cells and produce cytopathic effects (7). The source of Teschen disease virus for tissue culture was spinal cord and brain from experimentally infected pigs.

The present study deals with the isolation of viruses from nervous tissue and feces from outbreaks of a disease of suckling pigs occurring in Eastern Ontario, and reports the results of some serological comparisons with some other porcine enteroviruses.

Materials and Methods

Infective material and tissue culture inocula. The infective material was obtained from local field cases of the disease. In some trials brain and spinal cord served as the source of inoculum, while in others, fecal material was used.

Ten per cent suspensions of nervous tissue were prepared by grinding pieces of brain and cord in tissue culture maintenance medium with a glass tissue grinder. The suspensions were cleared of larger tissue fragments by centrifugation (1500 r.p.m. for 10 minutes) and further diluted with maintenance medium to provide a 1:100 final dilution of tissue. The suspension, with the addition of 100 units of penicillin and 100 micrograms streptomycin per ml, constituted the inoculum for tissue culture.

Feces were suspended in tissue culture maintenance medium by shaking approximately 1 gram of feces in 10 ml of fluid in a stoppered tube. Centrifugation at 2500 r.p.m. for 30 minutes deposited most of the fecal material, and the supernatant was further diluted 1:10 in maintenance medium to provide the inoculum. Antibiotics used included 100 units penicillin and 100 micrograms each of streptomycin, aureomycin and mycostatin per ml of inoculum.

Imported sera and viruses. For comparative studies, two strains of Teschen disease virus were used. These were identified as the Konratice and Reporyje strains, and one of them, the Konratice, was adapted to grow in tissue culture and employed in cross serum-virus neutralization trials (7).

Sera were prepared against each Teschen disease strain by the inoculation of susceptible pigs. A sample of Konratice strain antiserum was also obtained from Germany through the courtesy of Dr. Anton Mayr, Tubingen.

Sera and virus were obtained from Dr. A. O. Betts, School of Veterinary Medicine, Cambridge, England. They were identified as T52 and T80 and were associated with the polio-encephalomyelitis of baby pigs occurring in that country.

Tissue culture. All tissue culture studies were performed on monolayer cul-

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tures of porcine renal epithelial cells. Kidneys were removed aseptically from three week to two month old exsanguinated donor pigs. After removal of the capsule, portions of the cortex were excised and minced with scissors in a 0.25 per cent saline solution of trypsin.

The trypsinization procedure used was on some occasions the overnight method in the cold described by Bodian (8) or, on others, a modification of the Youngner technic (9). In the latter, trypsinization was carried out at room temperature with 2 or 3 changes of trypsin at hourly intervals. In either case, the cell harvest provided by trypsinization was concentrated by centrifugation at 1000 r.p.m. for 1 minute, and the packed cells diluted to 0.5 per cent (volume to volume) in cold growth medium. The suspension was filtered through 2 layers of gauze to remove fibrous tissue strands and pieces of kidney, distributed in tubes or flasks and incubated at 37°C. The tubes, which were placed initially in racks supported at a slight angle, were transferred at the time of inoculation to roller drums.

The growth medium consisted of 0.5 per cent lactalbumin hydrolysate (enzymatic), 0.1 per cent proteose peptone No. 3, and 10 per cent adult bovine serum in a solution of Hank's saline. The growth medium is referred to as medium "A".

For the initial part of the study the maintenance medium used during virus growth trials was of the same composition as medium "A" except that Earle's saline was substituted for Hank's, and it is referred to as "B" medium. Later, filtered bovine amniotic fluid (BAF) was used giving the advantages of simpler preparation, good maintenance properties and the avoidance of possible inhibitory substances in the bovine serum of medium "B" (10).

Serum-virus neutralization. Serum virus neutralization trials were carried out in tube monolayer cultures of the pig kidney cells. All sera were heated to 56°C for 30 minutes, then diluted in 2-fold steps from 1:10 in maintenance medium. To the serum dilutions was added an equal volume of maintenance medium containing 1000 tissue culture 50 per cent infections doses (1000 $TCID_{50}$). The dosage determination was made by applying the Reed-Muench formula (11) to the results of virus titration in tissue culture.

The serum-virus mixtures were incubated at 37°C for two hours, then three tissue culture tubes were drained of "A" medium and each inoculated with 1 ml of the mixtures. In effect, each tissue culture tube received 1 ml of inoculum containing a dilution of the serum on test, and 500 $TCID_{50}$ of virus.

The results of the tests could usually be read by the 4th day, but were occasionally held for six days. The interpretation of results was based on the presence or absence of cytopathic effects, and the serum titer end point recorded as the highest dilution that completely neutralized the virus.

Animal inoculations. Baby pigs were inoculated in some trials by trephination of the skull over the cerebrum and introduction of the inoculum by syringe and 25 gauge hypodermic needle into one hemisphere of the brain. In other trials the oral route was used. Intracerebrally the piglets were given 0.2 ml of inoculum while in oral exposure up to 2 ml of material was placed in the mouth.

The age of piglets used varied between one and six days. Litters selected included some which nursed the sow and some raised in isolation units as pathogen-free, colostrum-deprived orphans obtained by hysterectomy. In some trials, piglets were exposed orally to the virus as soon as they were born, then placed with the sow about 60 minutes after receiving the virus.

Results

Isolation of virus. A total of twelve attempts have been made to date to isolate virus from material obtained from eight outbreaks. Of these attempts, three were successful in that agents capable of promoting cytopathic effects were isolated and carried in successive passages in pig kidney tissue cultures.

The first agent isolated (PE-1) was from the brain and spinal cord of a baby pig, one of a litter of twelve which showed clinical evidence of encephalitis followed by subsequent death of all the piglets in the affected litter (Herd No. 2). Sixteen tubes of pig kidney cells were inoculated with a 1:100 suspension of tissue in "B" medium. Following four days incubation, four of the tubes showed small areas of rounded and refractile cells suggestive of early cytopathic effects. The other twelve tubes appeared normal and remained so

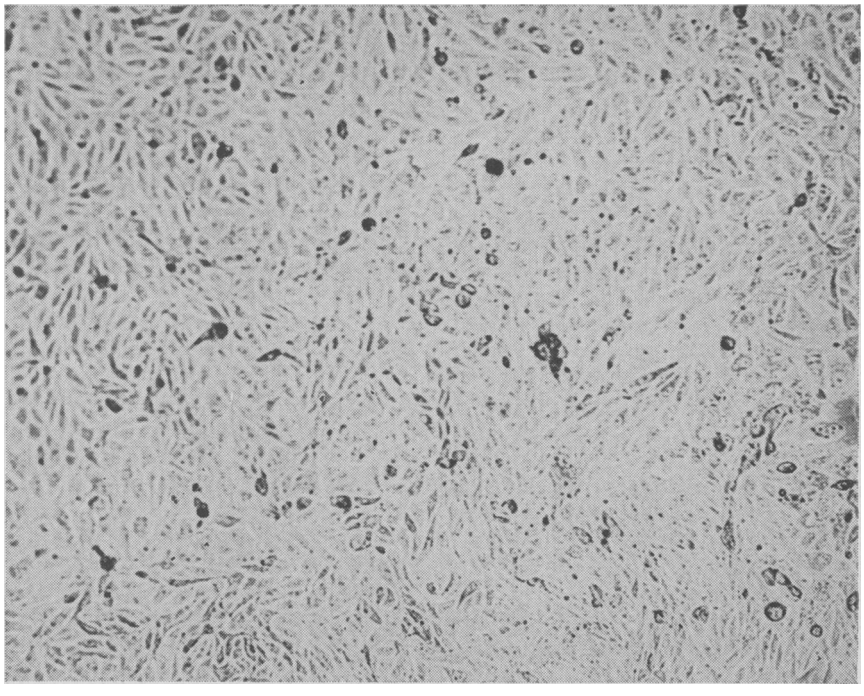

Fig. I—Uninoculated six day old culture of pig kidney. Unstained X100.

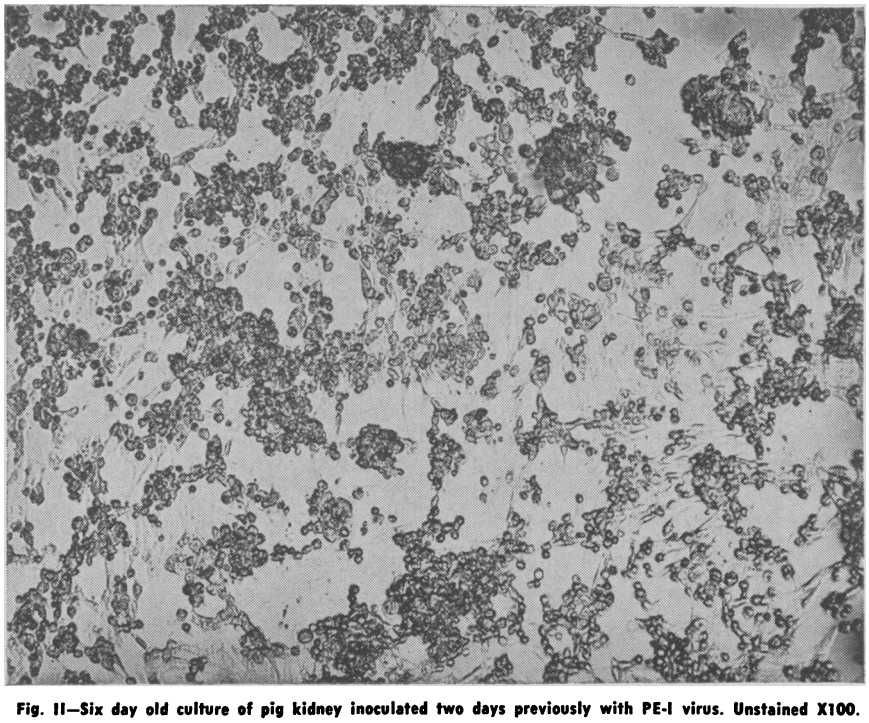

Fig. II—Six day old culture of pig kidney inoculated two days previously with PE-I virus. Unstained X100.

for eight days after which time they were discarded. A portion of pooled fluids harvested from the four affected tubes was diluted 1:10 in "B" medium and inoculated into a group of 10 fresh pig kidney cultures. In four days time all these second passage cultures showed large areas of cellular destruction. Subsequent passages up to the present thirtieth have been made at intervals of one to four days.

The second isolation (PE-2) was made from a pooled brain and cord specimen from two baby pigs. In this case (Herd No. 3), twelve of a litter of fourteen infected pigs died, nine within the first seven days after birth. The initial passage was made by inoculating twenty tissue culture tubes with a 1:100 tissue suspension in "B" medium. All the tubes appeared unaffected after five days incubation. In a second passage, two groups of ten tissue culture tubes each were used, one group in which the diluting maintenance medium was the usual "B" medium, and the other group in which bovine amniotic fluid (BAF) was used. Within four days a few of the cultures with BAF showed obvious foci of cytopathic changes, and from these tubes a third passage was made again using BAF as the maintenance medium. The second passage cultures in "B" medium remained normal in appearance, and were subsequently harvested and passaged on the seventh day of incubation, using BAF. Within four days this line too showed cytopathic effects. All subsequent passages up to the present twelfth have been made in BAF. Because of possible growth inhibition of the virus due to the bovine serum in "B" medium, the PE-1 strain of virus was cultivated in the presence of BAF from about the 12th passage onward.

The third isolation (PE-3) was from the feces of pigs which had recovered from a condition that appeared clinically and histologically the same as the others (Herd No. 4). In this case a previous trial with brain from an infected litter-mate had failed to reveal the presence of cytopathogenic virus in tissue culture. However, the feces when treated with antibiotics, and suspended in BAF, yielded a cytopathogenic agent in the first passage in tissue culture.

In each of these three cases, the agent isolated produced transmissible cytopathic effects in pig kidney cultures in from one to eight days, depending on the viral concentration. The first sign of infection in the tissue cells was an increase in cytoplasmic granularity. This was followed by a separation of the cells from each other, a rounding-up of the cytoplasm and increased refractivity. Finally there was detachment of the cells from the glass surface and fragmentation of the cytoplasm. Individual foci of necrosis occurred within twenty-four hours of inoculation and spread eventually to destroy the whole culture. See Figures 1 and 2.

Tissue culture fluids containing each of the isolated agents were tested for bacterial growth in thioglycollate medium, and medium designed for the growth of organisms of the pleuropneumonia group. In neither case were bacterial organisms detected.

In a series of trials, various concentrations of penicillin, streptomycin, aureomycin, mycostatin and neomycin failed to influence the growth or cytopathogenicity of the agents in tissue culture.

The agents were each readily filterable through a Seitz sterilizing pad with little loss of infectivity. Ten ml of BAF containing PE-1 agent were shaken with two ml of anaesthetic ether and held for twenty-four hours at 4°C. The ether was then removed by aspiration and evaporation at 37°C. Subsequent cultivation of the treated material showed the agent to be unaffected by ether. Similar trials with PE-2 and PE-3 viruses also showed them to be unaffected by exposure to ether.

It was concluded on the basis of filterability, resistance to antibiotics, resistance to destruction by ether, the absence of growth in bacteriological media and the pattern of cytopathogenicity in tissue culture that the agents isolated were viruses.

Infectivity trials. Five experiments involving a total of forty baby pigs were performed in attempts to assess the pathogenicity of the PE-1 strain of virus.

In experiment A, four of five baby pigs which had nursed for three or four days were given intracerebral inoculation of the ninth tissue culture passage of the virus. Pig A-5 was not inoculated but included as a contact control. After inoculation the pigs were removed from the sow and raised on a baby pig formula without sow's milk. No evidence of clinical disease occurred over the next thirty-four days. At the end of this time the experiment was terminated. The animals were bled for serum on

the nineteenth and thirty-fourth day. Histological examination of the brains revealed no evidence of encephalitis. Serological examination, however, showed that each pig, including the contact, developed a high titer of neutralizing antibodies. The results of this and all other experiments are summarized in Table I.

Experiment B involved six piglets inoculated intracerebrally with the twelfth tissue culture passage, and three uninoculated control pigs. In this trial the piglets were inoculated at the time of birth and before they were allowed to suckle. They were held for one hour after inoculation before being placed with the sow. Three of the inoculated pigs died, two on the second day and one on the fourth day, but on histological examination none of these showed evidence of encephalomyelitis. The remainder were bled thirty days after inoculation and the sera, when tested for antibodies, showed only low titers.

The third experiment, C, was similar to

TABLE I

Serological response in experimentally inoculated piglets to the PE-1 virus.

Experimental Litter	Animal Number	Inoculation Route	Pre-inoculation Titer	Post-inoculation Titer and days after Exposure	
A	1	Intracranial	20*	160 — 21 days	640 — 34 days
	2	"	20	320 — 21 days	640 — 34 days
	3	"	10	80 — 21 days	320 — 34 days
	4	"	20	80 — 21 days	160 — 34 days
	5	Contact	Neg.	40 — 21 days	80 — 34 days
B	1	Intracranial	Not Tested	died — 4 days	
	2	"	"	died — 2 days	
	3	"	"	died — 2 days	
	4	"	"	10 — 30 days	
	5	"	"	80 — 30 days	
	6	"	"	10 — 30 days	
	7	Control	"	Neg. — 30 days	
	8	"	"	Neg. — 30 days	
	9	"	"	Neg. — 30 days	
C	1	Intracranial	Not Tested	20 — 21 days	
	2	"	"	80 — 21 days	
	3	"	"	20 — 21 days	
	4	Control	"	Neg. — 21 days	
D	1	Intracranial	Not Tested	died — 2 days	
	2	"	"	40 — 28 days	
	3	"	"	80 — 28 days	
	4	"	"	died — 17 days	
	5	"	"	20 — 28 days	
	6	"	"	40 — 28 days	
	7	Control	"	Neg. — 28 days	
	8	"	"	Neg. — 28 days	
E	1	Control	Neg.	—	
	2	Oral	Not Tested	died — 2 days	
	3	"	"	died — 2 days	
	4	"	"	died — 2 days	
	5	"	"	160 — 20 days	
	6	"	"	died — 2 days	
	7	"	"	died — 2 days	
	8	Contact	Neg.	died — 9 days	
	9	Intracranial	Not tested	320 — 20 days	
	10	"	"	died — 17 days	
	11	"	"	died — 20 days	
	12	"	"	died — 3 days	
	13	"	"	died — 3 days	
	14	"	"	died — 1 day	

*Reciprocal of dilution of serum neutralizing 500 $TCID_{50}$ PE-1 virus in tissue culture.

TABLE II

Comparative antigenicity trials between PE-1, PE-2 and PE-3.

Virus	SERA E-1 (Pre-inoc)	E-8 (Pre-inoc)	E-5 (Post-inoc)	E-9 (Post-Inoc)
PE-1	10^{-7}*	10^{-7}	$< 10^{-1}$	$< 10^{-1}$
PE-2	10^{-6}	10^{-7}	$< 10^{-1}$	$< 10^{-1}$
PE-3	10^{-6}	10^{-6}	$< 10^{-1}$	$< 10^{-1}$

*Endpoint of titration in presence of 1:20 dilutions of sera, calculated to the nearest 10-fold dilution.

the second, involving a litter of four piglets, three of them inoculated intracerebrally and the fourth left as an uninoculated control. No signs of disease occurred in any of them. Neutralizing antibodies to the PE-1 virus were detected in the inoculated animals but not in the control.

In experiment D, six of the eight piglets were inoculated intracerebrally with dilutions of PE-1 virus, passage 24. Pigs D1 and D2 received 10^{-1} dilution of virus suspension, D3 and D4 a 10^{-2} dilution and D5 and D6 a 10^{-3} dilution. Pigs D7 and D8 were held as uninoculated controls. As in the preceding two trials, these piglets were inoculated shortly after birth and held for one hour before being placed with the sow. Pig D1 died on the second day, and histologically showed no evidence of disease. Pig D4 died on the seventeenth day. Histologically it showed perivascular cuffing with mononuclear cells involving both the gray and white matter in the cervical cord and one area of the medulla oblongata near the obex. The other areas of the central nervous system examined were normal. All the other pigs remained healthy and were challenged with Teschen disease virus intracranially twenty-eight days after the first inoculation. All of them became sick and died with Teschen disease. The sera taken prior to challenge with Teschen disease showed the presence of antibodies to the PE-1 virus.

In experiment E, fourteen colostrum-deprived piglets comprising portions of two litters were used. Six from one litter were given 0.2 ml of virus intracerebrally and one of the same litter was bled for pre-inoculation serum and held as a contact control. Six of the other litter received 2 ml of virus orally, and one piglet of this litter was bled out to provide a stock of pre-inoculation serum.

Pig E-14 of the intracerebral group died on the day following inoculation. Four more pigs died in the next twenty days, and the last one, E-9 was bled for serum and euthanasia and necropsy performed on the twentieth day. The contact control pig E-8, died on the ninth day of the experiment.

Five of the six orally exposed pigs died in two days time. The sixth was bled for serum and following euthanasia was necropsied on the twentieth day.

Prior to inoculation fecal samples were pooled from several piglets of each litter and tested in tissue culture for virus. None was detected. Feces taken from pig E-12 on the day it died, day three, yielded virus which was serologically identical to that inoculated. Feces cultured from pig E-9 on the fifth day and pig E-5 on the twentieth day also each yielded virus indistinguishable from the virus with which they were inoculated.

The pre-inoculation serum samples were negative for antibodies to PE-1 virus. The sera obtained from pigs E-5 and E-9 twenty days after exposure, on the other hand, demonstrated a high level of antibodies. These sera were selected for use in comparative antigenicity trials with other porcine viruses.

Some of the pigs in this experiment showed evidence of acute serositis on necropsy. There was excess peritoneal and pleural fluid and serofibrinous exudate covering the organs of the abdominal and pleural cavities. Bacteriological studies of the exudates and of fecal samples from some of these animals did not disclose the presence of pathogenic organisms.

Histopathological studies were conducted on the central nervous tissue of six of the pigs, including the contact control. Pigs E-8 and E-13 showed nothing of significance. Pigs E-9, E-10 and E-11 each showed lesions of mild encephalomyelitis which included perivascular cuffing, swollen epithelial cells and leucocytic occlusion of some vessels. Pig E-5, the sole remaining piglet exposed orally, showed a severe granulomatous ependymitis, but no evidence of encephalomyelitis. Bacteriological

cultures of spinal fluid and brain material from the pigs tested yielded no microorganisms.

In none of the inoculated pigs were any symptoms observed comparable in all respects to those seen in field cases of porcine encephalitis. A few showed weakness and some incoordination prior to death. Fifteen of the thirty-one inoculated piglets died, whereas none of the six uninoculated controls died. One of the two contact controls died. In experimental group E, the orally exposed piglets died soon after exposure and showed no prior symptoms. The other pigs of experiment E mostly exhibited weakness and loss of appetite for one or two days before death.

Serological Comparison of Isolated Agents

With antisera prepared in pigs against the PE-1 virus, the other isolated viruses were tested for comparative antigenicity. Neutralization tests were set up using a constant dilution, 1:20 of each serum against serial 10-fold dilutions of each virus. The results, shown in Table 2, indicate that all three viruses isolated are antigenically the same, and may be considered a single strain.

Cross Neutralization Tests With Other Porcine Agents

Table 3 summarizes the results of cross neutralization tests among the viruses and antisera of PE-1 virus, Teschen disease and the polio-encephalomyelitis of Betts, T80 and T52A. It is seen that there is no cross between PE-1 virus and Teschen disease virus.

The sera produced in colostrum deprived pigs, those of experiment E, appear to be specific for the PE-1 virus, and have no effect on T80 virus. However, T80 and T52A antisera each neutralized PE-1 virus at significantly high dilutions, suggesting a degree of cross reaction.

An unexpected reaction occurred in experimental litter A. The sera from all five pigs reacted the same way, but only that of pig A-1 is recorded in the table. Although there was a degree of protection afforded by the pre-inoculation sample against PE-1 virus, the post-inoculation samples showed a marked rise in titer. Contrary to this result, with T80 virus, the pre-inoculation sample exhibited a high titer which was reduced considerably by the time of termination of the experiment.

These results would suggest that litter A, which had suckled prior to inoculation, received colostral antibodies which reacted with the T80 virus. The reduction in titer after nineteen and thirty-four days may be explained as evidence of the normal disappearance of passive maternal immunity when the piglets were deprived of sow's milk. Immunity to the inoculated PE-1 virus increased within the same period as an active manifestation to the introduction of antigen.

Discussion

From the beginning of this study the available clinical and pathological evidence has pointed to the existence of an infectious encephalitic disease of baby pigs. Indeed, if the same condition is being observed, then the studies of Alexander *et al* (1) and Richard and Savan (2) would in-

TABLE III

Cross neutralization tests with sera and viruses of other porcine diseases.
Virus — 500 $TCID_{50}$ of each

Sera description	PE-1	Konratice	T80
Teschen-Konratice-Germany	< 10	320*	< 10
Pre-inoculation-Teschen-Reporyje	< 10	< 10	< 10
Post-inoculation-Teschen-Reporyje	< 10	640	< 10
Pre-inoculation PE-1 E-1	< 10	< 10	< 10
Pre-inoculation PE-1 E-8	< 10	< 10	< 10
Post-inoculation PE-1 E-5	160	< 10	< 10
Post-inoculation PE-1 E-9	320	< 10	< 10
Pre-inoculation PE-1 A-1	20	< 10	160
Post-inoculation PE-1 A-1 21 days	160	< 10	40
Post-inoculation PE-1 A-1 34 days	320	< 10	20
Porcine encephalitis (Betts) 52A	80	< 10	N.T.
Porcine encephalitis (Betts) T80	640	< 10	N.T.

*Reciprocal of serum dilution showing neutralization of virus — 1:10 was the lowest dilution tested.
N.T. Not tested.

dicate that an encephalitis was geographically quite widespread in Ontario pigs.

Since the disease has some resemblance to Teschen disease, one of the prime objectives of this study was to examine such a possible relationship. Clinically, although the Ontario disease bears some similarity to Teschen disease, it differs profoundly in pathogenicity. Available strains of Teschen disease virus produce encephalitis and death in Canadian pigs with no difficulty. Transmission trials with clinical material obtained from outbreaks of the Ontario disease have not shown the same degree of infectivity (12). The studies of Richard and Savan indicated too that the agent with which they were dealing produced infection in colostrum-deprived, orphan raised piglets, but not in piglets which had suckled.

Previous studies (7) showed that three available strains of Teschen disease virus, isolated from experimentally infected pigs, grew readily in tissue cultures of porcine kidney cells. These viruses induced antigenic responses in pigs resulting in the production of specific neutralizing antibodies.

In this study, virus strains have been isolated from brain material from two outbreaks of the disease, and from the feces of a pig involved in a third outbreak. All three of these isolated viruses were shown to be antigenically identical. Cross neutralization tests with Teschen disease virus and antisera have demonstrated that no serological relationships exist between the Ontario viruses isolated and Teschen disease virus. Further studies are required to assess what relationship, if any, exists between the polioencephalomyelitis virus of Betts and the Ontario virus.

The question of the causal relationship between the PE-1, PE-2 and PE-3 viruses and the disease as it occurs in the field remains to be answered. From the work to date it is obvious that the PE-1 virus, the only one so far studied extensively, is not highly pathogenic under the experimental conditions conceived up to now. However, there are a few facts which may be considered as evidence of a relationship to the disease. First of all, two of the three strains of virus were isolated from central nervous tissue of pigs showing symptoms of encephalitis. Although several reports exist of the isolation of orphan viruses from porcine feces (13, 14, 15), and these are apparently non-pathogenic parasites of the intestinal tract, it would seem suggestive that a virus present in diseased brain might play some part in the pathological processes within that brain. The third isolation, PE-3, was from the feces, showing that the viruses should probably be classed as entero-viruses. In this case the donor pig had recovered from the disease. In comparison, the T80 and T52 viruses of Betts were isolated from feces and tonsils of apparently healthy pigs and were later shown to be pathogenic for colostrum deprived orphan piglets.

Of the baby pigs inoculated with the PE-1 virus, at least four showed histological evidence of encephalitis, although it was of a mild nature. In addition, half of the inoculated pigs died during the course of the experiments whereas none of the uninoculated, non-contact pigs died.

In all cases, piglets surviving inoculation or exposure to the virus developed neutralizing antibodies. Pigs inoculated intrancranially were shown to shed virus in the feces as early as three days after inoculation. Serological studies of the field cases occurring in Eastern Ontario in relationship to Teschen, PE-1 and T-80 viruses will be presented in another paper.

Summary

Three isolations of virus were made from brain and feces of three outbreaks of encephalomyelitis in baby pigs in Eastern Ontario. The three viruses were shown to be antigenically identical to each other, and should probably be classed as entero- viruses. They are serologically different to the virus of Teschen disease and may be different to the viruses isolated in England by Betts. The causal relationship between the viruses isolated and the disease in baby pigs has not been satisfactorily established.

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The Therapeutic Use of Cysteamine in Bovine Ketosis

Thirty-three cows suffering from primary ketosis were treated with an aqueous solution of cysteamine hydrochloride. Two or three 750 mg. doses of cysteamine each contained in 250 ml. of sterile water were injected intravenously generally at three day intervals. The milk yield and appetite of 21 animals were recorded during the treatment. Blood samples were taken before and during the treatment from these animals and were analysed for serum levels of ketone bodies, glucose, pyruvate, alpha-oxoglutarate and citrate. All the initial samples showed a considerable deviation from the normal. All 33 cases of primary ketosis recovered completely after two or three injections with cysteamine. Concomitant with the disappearance of clinical symptoms was a restoration towards normality of the excessively high levels of ketone bodies, pyruvate and alpha-oxoglutarate and of the initially depressed values of glucose and citrate as ascertained from the analysis of the serum samples of 21 animals. The possible mode of action of cysteamine, possibly a precursor or a substitute for co-enzyme A was discussed.

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Composition of Beagle Dog Milk

A statistical study was made of the composition of the major calorie containing components of beagle dog milk during the third through the fifth week of lactation. The dogs used in the study were obtained from a parasite and disease free colony of pure bred beagles maintained for life span studies. The dogs were of similar age and body weight and were in a period of well established lactation (two to five weeks post-partum). Each dog had littered at least once prior to the experiment. During each twenty-four hour collection period the dogs were separated from their litters. Milking was accomplished by hand following intravenous injection of five international units of synthetic oxytocin. Milking required less than five minutes. Repeated injections of oxytocin failed to increase milk yield significantly. The average milk production during each three hour interval was 96 cc. The basal ration was made up of 41% meat scraps, 34% toasted grain flakes, 6% fish meal, 6% soybean meal, 3% tomato pulp, 1.6% alfalfa meal, 1.6% meat meal, 1% liver meal, 1% dried milk powder, 0.5% dried egg, 1.2% vitamin mixture, and 0.5% salt mixture. This ration was moistened with water and supplemented with freshly ground beef (60 to 20 to 20) prior to feeding. The mean values of analysis of the bitches' milk were: 26% total solids, 13% fats, 3.3% lactose, and 9.8% protein. Total solid content was found to vary with fat content.

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